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<p>Two of the three segments of the planned studies, adhesive skin wound augmentation (ASWA) and burn wound healing (BWH) are underway. Plans for the third segment, hemostasis, have been finalized. The studies on ASWA are practically complete and they include: (1) polymer synthesis and characterization of primary gel-formers; (2) preparation and evaluation of candidate formulations; and (3) conducting the animal studies and completing data analysis. Studies on BWH are in progress and they include (1) preparation and evaluation of candidate formulations, and (2) initiation of the animal studies. Results of the study on the effect of gel-forming formulations on skin wounds indicate that (1) using gel-formulations without antibiotics can lead to improved wound healing and increase in wound strength regain with partial reliance on mechanical stapling, and (2) high concentrations of the antibiotic vancomycin compromises the wound healing.</p>			
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S. W. Shulsky

PI - Signature

12/13/96
Date

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A. INTRODUCTION

Many approaches are being used for treating traumatic and burn wounds such as those encountered in battlefield injuries and burns. However, constraints such as infections, excessive bleeding and/or extreme tissue sensitivity made the treatment of these wounds especially challenging. Thus, the primary objective of this nine-month program is to develop a bioabsorbable (or simply absorbable) hemostatic tissue adhesive with most, if not all, of the following attributes: (1) it can be extruded easily from a syringe as a viscous liquid formulation; (2) the extruded liquid adheres to the tissues and provides sufficient bond strength to keep approximated ends at the wound site in position during healing; (3) the extruded liquid transforms into a gel form at an irregular wound site to allow for 2-4 week residence time and modulates the oxygen and water vapor transmissions; (4) the extruded system before and after gel formation should be mechanically and chemically compatible with injured tissue and any exposed nerve endings; (5) the formulation can be used for the controlled delivery of antibiotics such as vancomycin; and (6) the selected formulations do not interfere with, and preferably accelerate, wound healing. As a secondary objective, the developed formulations can eventually be used clinically to deliver growth factors for accelerated wound healing. The major milestones of the proposed study include (1) the preparation of several candidate liquid polymeric systems from selected, newly synthesized proprietary gel-forming absorbable polymers; (2) screening the candidate systems for gel-formation, hemostasis and adhesion to animal tissue; (3) evaluation of a selected candidate from "2" for *in vitro* absorption and release profile of vancomycin; and (4) evaluation of two selected candidates for efficacy as a hemostatic tissue adhesive using the proper animal models.

B. MATERIALS AND METHODS

B.1. Materials

Monomers, pre-polymers, and key chemical reagents used in this segment of the program were purchased from the respective supplier as listed below.

<u>Chemical</u>	<u>Supplier</u>
Glutaric anhydride	Aldrich Chemical Company
Glycolide	NORAMCO (J&J)
dl-Lactide	Purac
Polyethylene glycol 400	Aldrich Chemical Company
Polyethylene glycol 1000	
Stannous octoate	Sigma Chemical
Vancomycin hydrochloride	Sigma Chemical

B.2. Methods

B.2.1. Polymer Synthesis and Characterization

General Polymerization Method--All copolymers used in this segment of the program were prepared by end-grafting polyethylene glycol 400 (PEG 400) or polyethylene glycol 1000 (PEG-1000) with a mixture of glycolide and dl-lactide in the presence of a catalytic amount of stannous octoate. The PEG-400 or PEG-1000 was used as the initiator for the ring-opening polymerization. The ring-opening polymerization (end grafting) was conducted and the final product was isolated as described by Shalaby (1995).

Polymer Characterization--Polymers made as described in Section B.2.1. were characterized for (1) chemical identity using FT-infrared (FTIR) spectroscopy--then, liquid polymers (neat cast from acetone) were analyzed on a Perkin-Elmer Paragon 1000; (2) composition of the copolymeric chains using nuclear magnetic resonance (NMR, both proton and ^{13}C)--the polymers were examined in CDCl_3 on a Bruker -300 NMR spectrophotometer; (3) molecular weight and molecular weight distribution of the polymers by gel permeation chromatography (GPC)--the polymers were analyzed as solutions in tetrahydrofuran on a Waters Associate GPC unit; and (4) thermal transitions at or above room temperature using differential scanning calorimetry (DSC)--about 5 mg samples were cooled to -20° and then heated under nitrogen to 150°C at $10^\circ/\text{min}$ heating rate on a Perkin-Elmer DSC-6. A syringe with an 18 gauge needle was used to determine, qualitatively, the fluidity of the individual polymers or their mixtures at 25°C and 37°C .

Preparation of and Characterization of Carboxy-Terminated Gel-Formers--A liquid gel-former made by end-grafting mixtures of dl-lactide/glycolide mixtures onto liquid PEG-400 was used. Typically, the liquid polymer, which is hydroxy-terminated, is treated with an equivalent amount of glutaric anhydride to esterify the end-groups and form carboxylic groups at both ends of the chain. The reaction is carried out by heating the mixture at about 100°C for 30 min., 110°C for 40 min, then at 120°C for 40 min. The product was then heated under reduced pressure (about 0.1 mm Hg) at 120° to remove traces of unreacted anhydride. The presence of carboxylic end-groups was confirmed by titration in conjunction with IR spectroscopy and GPC (for molecular and molecular weight distribution).

B.2.2. *In Vitro* Evaluation of Candidate Gel-Formers

Different combinations of the original gel-formers having different chain structures, hydrophilicity, and solubility were evaluated and rated on a scale of 1 - 5 in the different test categories using 5 and 1 as the most and least desirable, respectively.

Gelation Time--A 0.5 ml aliquot of the gel-former was extruded from a syringe needle into 5 ml buffer solution at pH 7.2 and 25°C and time required to form a coherent 3-dimensional mass was noted.

Adhesive Property--At this stage of the program, the adhesive property was determined in terms of the ability of the gel-former to adhere to the walls of a glass vial at 25°C in presence of phosphate buffer at pH of 7.2. Thus, a 0.5 ml of gel-former was extruded from a syringe

needle into 20 ml of a buffer solution, left to equilibrate for 5 min. The vial is then shaken for 10 seconds and resistance to dislocation of the gel mass from the bottom of the vial is used as a measure of its adhesive property.

B.2.3. *In Vitro* Absorption and Release Studies of Drug Loaded Formulations

In Vitro Absorption--Relative rates of absorption of four selected gel-formulations containing 5 and 10 percent vancomycin were determined in terms of time required for practically complete dissolution at 50°C in a phosphate buffer solution (0.5 g. formulation in 50 ml buffer) in a shaker incubator at 50°C.

Release Profile of Drug-Loaded Formulation--In a typical experiment, the gel-former is mixed with 5 or 10 percent vancomycin. The drug-loaded polymer is transferred to a continuous-flow cell attached to a peristaltic pump. The buffered phosphate solution was passed tangentially by the surface of the drug release systems. Samples of the effluent buffer were collected at regular intervals over a period of 15 days. The drug content of vancomycin in these samples was determined using an HPLC method developed at Poly-Med. This method calls for use of an acetonitrile/water mobile phase and a C₁₈ column.

B.2.4. Animal Studies

To date, the study on (1) adhesive properties and skin wound augmentation (ASWA) is practically completed; (2) burn wound healing is progressing; and (3) the hemostatic property is yet to be initiated (using recently finalized plans).

Adhesive Skin Wound Augmentation (ASWA)--The study was conducted using hairless rats. A selected formulation containing 2 percent vancomycin and its placebo were used in the study. In a typical experiment, two skin incisions were made on opposite sides of the spine of a hairless rat. The incision was closed by stapling, using the traditional number of metallic staples. The second incision was closed using less than half the number of staples and the wound was then covered completely with the gel-former (with or without vancomycin). All staples were then removed at 10 weeks, post-operatively. At the conclusion of a 3-week period, the animals were sacrificed, the area about the healed incision was removed and cut to determine the wound breaking strength. This was done using a Satec universal testing machine. Details of the animal protocol are given in Appendix A. The augmented wound breaking strength was measured following a similar protocol to that described by Linden and Shalaby (1996).

Burn Wound Healing (BWH)--Prior to conducting the wound healing experiment, an animal model was developed. This entailed the construction and calibration of an electrically heated copper stamp for inflicting a third-degree burn in the skin of a hairless rat. The stamp dimensions are 1 x 1 cm and can be used to provide a 100°C surface burn. The 100°C surface, after being immersed in boiling water for 10 sec. was shown to create a second-degree burn by contacting the skin for 10 sec. The surface temperature of the dry metallic stamp (prior to immersion in water) was measured using a thermometer. In determining contact time, a pilot study was conducted where the skin was exposed to the stamp for time intervals ranging between 5 and 20 seconds. This was followed by histological evaluation of the burn site. And burn wounds displaying a second-degree burn were inflicted during a contact time of 10 sec.

To test the effect of a selected gel-formulation, the same gel used earlier in wound strength regain was chosen for the burn wound study. In a typical experiment, one burn was inflicted on each side of the spine. One of these burn wounds was allowed to heal as a control. The second burn wound was then treated with (1) a drug-free gel-former; (2) a gel-former containing 0.2 percent vancomycin; and (3) a gel-former containing 1 or 2 mg. of RGDS/ml of gel-former. In all cases, the treated burn wound was fully covered with the specific gel-former (using about a 0.5 ml aliquot). At this point the study is in progress and additional details will be reported at the conclusion of Phase I. Meanwhile, details of the animal protocol are provided in Appendix B.

C. EXPERIMENTAL RESULTS AND DISCUSSION

C.1. Synthesis and Characterization of Primary Gel-Formers

Five primary gel-formers (GF) having the composition shown in Table I were prepared and their composition and molecular dimensions coincided with those expected. The polyester copolymer was made of GF-D, carboxy-terminated, to produce GF-E.

Table I. Compositions of the Primary Gel-Formers and Mixtures Thereof

Primary Gel-Formers	GF-A	GF-B	GF-C	GF-C	GF-E*
● Composition of Primary GF's					
PEG: Type (wt%)	400(85)	1000(30)	400(30)	400(20)	400(20)
Polyester: L/G (%)	60/40(15)	80/20(70)	60/40(70)	60/40(80)	60/40(80)
● Composition of Mixtures made of primary GF's (%GF)					
GF-I	30	70	-	-	-
GF-II	26	-	74	-	-
GF-III	30	-	-	70	-
GF-IV	40	-	-	60	-
GF-V	17	-	27	56	-
GF-VI	10	-	10	-	80
GF-VII*	-	-	25	75	-

* Carboxy-Terminated

Viscosity and solubility characteristics of the individual primary GF's were considered less than optimal for use, separately, in any of the segments of Phase I. This is because each of these GF's displayed relatively extreme property in terms of hydrophilicity, gelation time, adhesiveness and/or absorbability. Therefore, it was decided to prepare and study the properties of selected mixtures of the primary GF's as planned in the original program proposal. GF-D was carboxy-terminated (to form GF-E) and its equivalent weight, as determined by titration, was comparable to the expected value of about 1200 Da.

C.2. Preparation and Properties of Mixed Gel-Formers

Seven combinations of the primary gel-formers were prepared and their compositions were described in Table I. Based on the properties of these mixtures, in terms of gelation time and adhesion to polar substrates (e.g., Pyrex glass surface), four most promising systems (III, V, VI, and VII) were chosen for conducting additional studies (as in Section C.3.).

C.3. Preparation Properties and Selection of Candidate Formulations for *In Vivo* Studies

Effect of Composition on Gel Quality and Drug Release Profile--Gel-formers (GF-III, GF-V, GF-VI, and GF-VII) described in the previous section were selected to load with 10% vancomycin; an additional sample of GF-VII was loaded with 5% vancomycin. The release profile of these systems over 15 days was monitored and the results indicate that (1) using GF's based on PEG-400 copolymers are most promising in terms of more controlled, slow release; (2) having high fractions of the high molecular weight GF-D is essential for attaining acceptable gel mechanical integrity; (3) incorporating GF-A and/or GF-B enhances the adhesive property of the gel; (4) using carboxy-terminated components do not slow the drug release rate; and (5) having a high drug concentration of 10% does not compromise the flow properties or the drug release profile of the formulation.

Effect of Composition on Relative Absorption--Results of the absorption study in a phosphate buffer at pH 7.2 and 50°C indicate that the absorption of the four formulations having 10% vancomycin decrease in the following order:

$$\text{GF-III} > \text{GF-V} > \text{GF-VII} > \text{GF-VI}$$

Identification of Different Formulations for the *In Vivo* Study and Rationale--For the **adhesive skin wound augmentation** study, it was decided to use the polymeric carrier of GF-V for its optimum adhesive and gel-forming qualities. Vancomycin loading of a relatively high concentration (compared to common topical formulations) of 2% was selected to maximize the effect of this antibiotic on the healing process. It was expected that antibiotics may interfere with the wound healing process. However, this protocol was followed since a key potential clinical application of the gel-formers is expected to be associated with infected wounds (as in battlefield situations). The role of high concentrations of antibiotics needed to be explored in a quantitatively evaluated animal model.

To determine the effect of the composition, both the polymeric carrier and active components on **burn wound healing**, the following compositions were chosen for the cited reasoning:

(1) The polymeric components of GF-V were selected for their optimum adhesiveness and gel-forming quality. In addition, using the same system in wound augmentation and burn wound healing will allow for a comparative evaluation of the role of the placebo formulation in two different procedures.

(2) A 0.2% loading of vancomycin was used to minimize any possible compromise of the healing process. This concentration is comparable to those used in many topical antibiotics applications. Such loading represents only 10 percent of the concentration used in the adhesive and wound augmentation procedure, since the effect of the gel-formulation in the burn study will be qualitatively assessed and stressing the biological system was not necessary.

(3) Arg-Gly-Asp-Ser (RGDS), an oligopeptide carrying an adhesion site of the adhesion protein fibronectin (i.e., tripeptidyl sequence RGD), was selected in order to study its effect on modulating the burn wound healing. The role of RGDS in woud healing and relevant biological events has been noted by a number of authors (Garcia et al., 1996; Holland et al., 1996; Streeter & Reese, 1987). Although the effective dose of RGDS varies depending on application site, a 1 mg/ml of gel-former dose was chosen as a moderate- to high-dose. Meanwhile, in an attempt to assess the effect of RGDS concentration, a 2 mg/ml dose was also used.

Toward determining the efficacy of gel-formers as hemostatic agents, one basic polymeric carrier, namely GF-V, will be used for its aforementioned attributes and to allow for comparing its performance in the three segments of the animal study. Additionally, the following formulations will be used for the cited reasoning.

(1) The carrier of GF-VII will be used to determine the effect of carboxylic end groups on the hemostatic property of the gel-former.

(2) The carrier of GF-VII will be mixed with zinc acetate (or calcium acetate) and ferrous lactate to determine the effect of divalent and trivalent ions on the hemostatic properties.

(3) The carrier of GF-VII will be mixed with RGDS to determine the effect of the latter on the hemostatic properties of the gel-former.

C.4. Adhesive Skin Wound Augmentation Study

This study was pursued as per the animal protocol in Appendix A. Main segments of the study and the pertinent results are summarized below.

C.4.1. Preparation of Placebo and Active Gel-Formers

Gel-Former Composition

<u>Polymer</u>	<u>Weight Percent</u>
GF-D	56
GF-C	27
GF-A	17

The above gel-formers were mixed with 2% vancomycin to produce GF-V.

Two sets of gels were prepared: GF-V without vancomycin was labeled Placebo Gel; GF-V with vancomycin was labeled Active Gel. These compositions were prepared and loaded in a laminar flow hood, into 1 cc syringes for surgery.

C.4.2. Animal Surgery and Results

Subjects--Twelve CD hairless rats, 6/group were used in the study. Each rat was injected with 0.05 mg/kg Buprenex and 0.50 mg/kg Acepromazine maleate. Once the rats appeared sedated, anesthesia was maintained with 2% isofluorane.

Procedure--Each rat was shaved, scrubbed thoroughly with Nolvasan Scrub, and wiped down with rubbing alcohol. Two 5 cm incisions were made 2 cm lateral to the dorsal midline beginning at the level of T-11. One incision was closed using nine metal staples placed 0.5 cm apart. The second incision was closed using four metal staples placed 1 cm apart with 1 cc of gel applied on the incision. The rats were placed in cages lined with paper for recovery from anesthesia. Rats received buprenorphine via subcutaneous injection every 8 to 12 hours for 24 hours after surgery.

Observations--From the time of surgery until the staples were removed, several of the animal care technicians noted that the control incision with nine staples looked puckered and bunched up. They felt the incision with only four staples looked better but could not say if this was due to the gel or to fewer staples.

At one day after surgery, one rat from the placebo group was euthanized because it had removed several staples and left a gaping wound that could not heal. Skin samples from this animal were used to validate mechanical test methods used at the end of the study.

Six days after surgery, one of the rats in the Active Gel group removed several staples and left a gaping wound that could not heal. This rat was euthanized. Overall, the other rats appeared to be healing well.

Ten days after surgery, all staples were removed. All incisions appeared to be healing, and all rats were gaining weight. Very superficial scabs were noted on several rats.

Euthanasia and Preparation for Wound Breaking Strength Measurement--The incisions on all rats appeared to have healed well. Scabs were still present on several rats. All rats were euthanized in a CO₂ pre-charged chamber except for two euthanized by heart puncture as noted below. Skin was collected from around the incision sites and marked with suture loops at the end closest to the head. Each skin sample was placed in a specimen jar containing saline. Upon skinning the animals, the scabs were noted to be very superficial with an increased vascularity underneath. A lump was found on one of the rats on the Placebo Gel test side. The lump was not near the incision, but rather lower, near its stomach. The lump was removed and placed in formalin for histopathology.

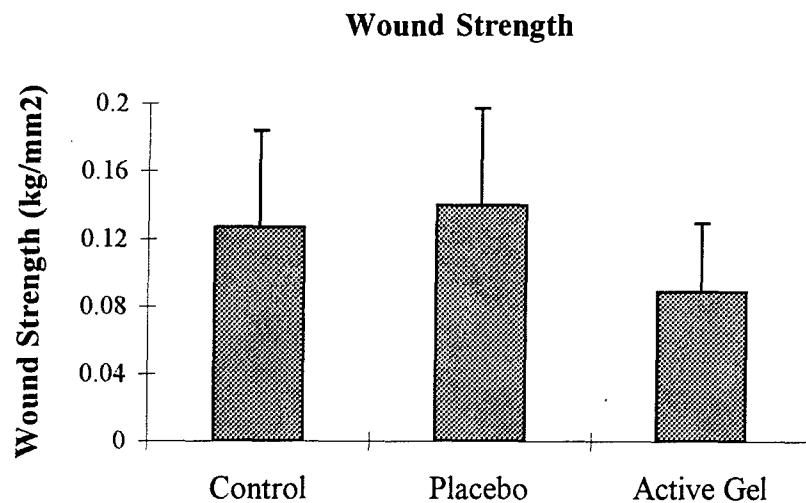
Two rats, one without any scabs at all, and one with the largest scab, were given ketamine/xylazine and euthanized via heart puncture for blood collection in order to get a complete blood count (CBC) to test for infection. A culture swab was also taken from the

scabbed skin. Finally, a tissue sample of the scab and one of the skin without any scabs were taken and placed in formalin for histopathology.

Wound Strength Testing--Testing was conducted within 12 hours of euthanasia. Samples were transported to the packaging lab where each incision was cut into five test specimens measuring approximately 1 cm x 10 cm. The width and thickness of the healed incision were measured for each specimen.

Samples were tested for wound strength using a Satec T10000 at a ram rate of 50 mm/min. Samples were tested to failure which was defined as an 80% drop in force. The data were recorded and a graph of load vs. displacement was printed. The wound strength was calculated as the maximum force applied over the area of the incision site.

Results of Wound Strength Testing--The results of the histopathology, culture swab, and CBC have not been received yet. Results on wound strength are shown below.



C.5. Burn Wound Study

The pilot study to determine the animal protocol for achieving a second degree burn was completed. The main study was initiated and is now in progress. The animal protocol followed in the study and is provided in Appendix B. Highlights of the ongoing study, as of December 6, 1996, are summarized below.

C.5.1. Preparation of Placebo and Active Gels

The placebo gel-former was the same as that used in the skin wound augmentation study. This, the polymeric carrier of GF-V (GF₁), and three other gel-formers were prepared and transferred into 1 ml syringes in a laminar flow hood. The three other formulations were based on the placebo and (1) 0.2% vancomycin (GF₂); (2) 1 mg/ml RGDS (GF₃); or (3) 3 mg/ml RGDS (GF₄).

C.5.2. Animal Preparation and Treatment

Subjects--Ten CD hairless rats.

Animal Treatment and Procedure--Each rat was injected with 0.05 mg/kg Buprenex and 0.50 mg/kg Acepromazine maleate. Once rats appeared sedated, anesthesia was maintained with 2% isofluorane. The hot stamp used in this study was a modified soldering iron with a 1 cm² copper plate attached to the end. The stamp was connected to a rheostat, which was set at 60%. The soldering iron was heated while in contact with a thermometer until the thermometer reached 100°C. The stamp was then immersed in boiling water for ten seconds. The stamp was immediately placed on the anesthetized rat, at approximately 1 cm lateral to the dorsal midline just below the shoulder blades. The stamp was held on the rat for ten seconds. Based on the results of a pilot study, this burn protocol results in a second degree burn of the rat. Each rat received two burns.

Three to five minutes after burning, the burn areas were measured. The left burn on each rat received 1 cc of one of the gel formulations. Collars (Elizabethan) were placed on the rats' necks to prevent them from accessing the burn wounds.

C.6. Problem Areas and Corrective Measures

Identified Problems

1. Toward achieving small differences in wound healing of augmented skin wounds, the use of sutures was thought to compromise detection of such differences.

Corrective Measure--Easy-to-apply metallic staples were used to approximate the wound edges.

2. Two of the augmentation study rats "Picked" most of the staples at 1 and 6 days, post-operatively, leading to wound gaping. These animals were euthanized.

Corrective Measure--Elizabethan collars were purchased and installed on the rats used in the burn wound healing study.

3. Adhesion measurement of the gel-former to wet animal tissue was not conducted due to inavailability of the proper load cell.

Corrective Measure--A new tensile tester with a low load cell was located and will be used.

4. Permeability data for gel-formers on fabric could not be obtained in a timely fashion prior to conducting the skin augmentation study. This was due to inavailability of properly functioning equipment for the constructed composites.

Corrective Measure--New gel-fabric composites will be prepared and tested.

Anticipated Problem

The gel-formers similar to those planned for studying the hemostatic properties of gel-formers, may be too viscous for a timely administration to bleeding tissue.

Corrective Measure--In the pilot study, the role of viscosity and wettability will be emphasized and dilution of gel-formers with GF-I (a low viscosity material) may be explored.

D. CONCLUSIONS

1. Representative members of the subject family of absorbable gel-formers are viable candidates for use as (1) a surgical adhesive in conjunction with limited number of surgical staples; and (2) a wound cover to accelerate wound healing.
2. High concentrations of antibiotics may compromise wound healing and timely regain of wound strength.
3. The development of a precise burn wound model is feasible using hairless rats.

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APPENDICES A AND B

CLEMSON UNIVERSITY
PROTOCOL FOR USE OF LIVE VERTEBRATES

(SUBMIT THE ORIGINAL AND TWO COPIES TO THE ANIMAL RESEARCH COMMITTEE COORDINATOR, 301H BRACKETT HALL.)

PRINCIPAL INVESTIGATOR	<u>Dr. Shalaby W. Shalaby</u>	PHONE: <u>646-8544</u>
P.I. OF ACTIVITIES AT CLEMSON	<u>Dr. J. David Gangemi</u>	PHONE: <u>656-1440</u>
COLLEGE/UNIT	<hr/>	
DEPARTMENT	<u>Greenville Hospital System/Clemson University Biomedical Cooperative</u>	
PROJECT TITLE	<u>Extrudable Gel-Forming Bioabsorbable Hemostatic Tissue Adhesives for Traumatic and Burn Wounds: Part II—Tissue Adhesives</u>	

SIGNATURES

As principal investigator, co-investigator, farm manager, or department head/chair, I verify that: (1) the information herein is true and correct and that I am familiar with and will comply with the legal standards of animal care and use established under federal laws, state laws and guidelines, as well as university policies; (2) the proposal has received approval for scientific merit by peer review; and (3) the activities do not unnecessarily duplicate previous experiments. I agree to advise the Animal Research Committee in writing of any changes in the procedures or personnel involved in this project. Such changes will not be implemented until committee approval is obtained. If a change in principal investigator becomes necessary, the committee will be notified immediately.

PRINCIPAL INVESTIGATOR	<u>L. Shalaby</u>	DATE: <u>4/23/96</u>
P.I. OF ACTIVITIES AT CLEMSON	<u>J. David Gangemi</u>	DATE: <u>5/1/96</u>
COLLEGE/UNIT	<hr/>	DATE: <hr/>
DEPARTMENT CHAIR	<u>J. David Gangemi</u>	DATE: <u>5/1/96</u>

Your signature as attending veterinarian verifies that you have: (1) reviewed and are familiar with this proposal; (2) consulted with the principal investigator(s) regarding any surgical procedures and any other procedures that may result in pain or distress; and (3) agree to perform all duties of the attending veterinarian in accordance with The Animal Welfare Act.

ATTENDING VETERINARIAN	<u>H. E. Farris, Jr.</u>	DATE: <u>5-6-96</u>
(SIGNATURE)		

TYPED NAME	<u>Harold E. Farris, Jr.</u>	PHONE: <u>656-5034</u>
-------------------	------------------------------	-------------------------------

FOR ARC USE ONLY		
ASSIGNED TO:	<input type="checkbox"/> FULL COMMITTEE	AUP NUMBER <u>96-054</u>
	<input checked="" type="checkbox"/> DELEGATED	
USDA CATEGORY	<hr/>	AMENDMENT NUMBER <u>—</u>
INSTITUTIONAL CATEGORY <u>A-2</u>		

ARC APPROVAL:		
CHAIR SIGNATURE	<u>Elizabeth Runkel</u>	DATE: <u>5-29-96</u>

GENERAL INFORMATION

1. SUBMISSION TYPE

- New
- Addendum
- Renewal/Continuation (Previous Protocol Number) _____

2. CLASSIFICATION

- Research
- Teaching
- Demonstration
- Farm Management

3. FUNDING SOURCE(S) & GRANT APPLICATION TITLE(S), IF APPLICABLE

U.S. Army Medical Research & Materials Command

Fort Detrick, Frederick, MD 21702-5012

4. COURSE NUMBER(S) AND TITLE(S), IF APPLICABLE

N/A

5. PROJECTED START DATE June 1996 PROJECTED END DATE February 1997

6. ABSTRACT

PHS policy requires submission of an abstract including the items listed below. The abstract must be submitted as Attachment 1 and should not exceed two (2) pages. The abstract must be written to ensure comprehension by non-scientists (preferably at high-school level). The following must be included:

- objectives of the research or teaching activity
- species and number of animals
- schedule of the course or the study procedures performed during each phase
- benefits, outcome and results expected

7. FLOW SHEET

A flow sheet is required and must be submitted as Attachment 2. Relative information (i.e., numbers of animals, experimental manipulations) should be provided on a flow sheet diagram. The ARC member should be able to follow each manipulation of the animal from initiation to termination.

8. PHS POLICY

PHS policy requires a copy of *Section F from NIH applications* and a copy of all animal methods sections from the proposal. Please provide as an attachment, if applicable. DO NOT SUBMIT THE ENTIRE APPLICATION.

GENERAL INFORMATION
DUPLICATE PAGE AS NEEDED

9. DESCRIPTION OF ANIMALS (COMMON NAMES REQUIRED)

SPECIES: Rat		
BREED/STRAIN: CD Hairless		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input checked="" type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input checked="" type="radio"/> FEMALE	3 mos.	250 g
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments
12	12	

SPECIES:		
BREED/STRAIN:		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input type="radio"/> FEMALE		
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments

SPECIES:		
BREED/STRAIN:		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input type="radio"/> FEMALE		
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments

GENERAL INFORMATION

10. SOURCE OF ANIMALS

- Commercial Vendor Charles River Company, Brookline Massachusetts
- Captured from Wild (A copy of the permit MUST be attached.)
- Transferred from Another Protocol; provide number _____
- Bred or reared at Clemson
- Donated to become Clemson property
- Other: _____

11. METHOD OF ANIMAL IDENTIFICATION

- Cage Cards
- Ear-Punch
- Collars
- Leg Bands
- Wing Tags
- Other Tags
- Tattoos
- Electronic
- Branding (Freeze)
- Branding (Hot-Iron)
- Other: _____

12. DISPOSAL OF ANIMALS AFTER COMPLETION OF STUDY/PROCEDURE

- Euthanized by methods outlined in the Euthanasia section of this protocol
- Returned to wild
- Returned to production/breeding unit
- Sold
- Transferred protocol number: _____
- Slaughter with conformation to the Humane Slaughter Act
- Other: _____

ALTERNATIVES TO THE USE OF ANIMALS - The Animal Welfare Act requires that the Animal Research Committee must ensure the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to animals, and has provided a written narrative description of the methods and sources.

13. What is the justification for using live animals rather than alternative means of achieving the research goals?

The proposed study involves healing and strength regain of surgical wounds. Therefore, there is no *in vitro* model to substitute for the *in vivo* one.

GENERAL INFORMATION

14. You are required to conduct a literature search to determine that either (1) there are no alternative methodologies by which to conduct this study, or (2) there are alternative methodologies, but these are not appropriate for your particular study. What procedures, specific databases, and sources did you use to determine that non-painful alternatives were not available or appropriate?

- AGRICOLA
- Animal Welfare Information Center
- Biological Abstracts
- Index Medicus
- National Agricultural Library *Phone: (301) 504-6212*
- Contacted colleagues
- Other: MED-LINE, Chem Abstracts, National Medical Library

What were your findings with respect to alternative methodologies?

No alternatives exist.

15. Why have you selected the particular species proposed in this project?

Lowest possible form of vertebrate.

16. Provide an explanation of how the numbers of animals to be used were derived. Numbers should be based on scientific and statistical requirements to achieve objectives. These numbers must be consistent with those used in the flowsheet. If statistical assistance is needed, contact the Office of Experimental Statistics at 656-3028.

To test the tissue adhesive properties of Gel Formulations III and IV, six mice will be used for each formulation, five for testing and one as a spare. Five mice for testing the tissue adhesive properties represents the minimum number required to obtain statistically reliable data.

DUPLICATION OF RESEARCH - The Animal Welfare Act requires that the principal investigator provide written assurance that proposed research is not unnecessarily duplicative.

17. Does the proposed research duplicate any previous work?

- Yes
- No

17a. If no, what procedures and sources did you use to determine that the proposed research does not duplicate previous work?

MED-LINE, Chem Abstracts, and the National Medical Library

17b. If yes, provide justification for the need to duplicate previous work.

N/A

QUALIFICATIONS OF PERSONNEL
(Duplicate this page as needed.)

Carefully review the Qualifications of Personnel instruction section located in the front of this form. Complete the following for people who will conduct procedures using animals (especially surgery, anesthesia, pre- or post-operative care, or euthanasia). An occupational health program is mandatory for personnel who work with laboratory animal facilities or have animal contact. The ARC will provide advice and training on all of these procedures if the personnel listed have no previous relevant experience.

List individuals who will have animal contact in association with this protocol and provide the date of their enrollment the Clemson University occupational health program. If individuals listed in this section are not enrolled, they must contact the Nursing and Wellness Center at 656-3076 to enroll or to sign a waiver of exclusion. Individuals not enrolled cannot participate in the study and approval will not be given until enrollment is verified.

FULL NAME: Research Services Personnel

TITLE: _____

Clemson University Animal Care and Use Seminar attendance

Date attended: _____
 Date scheduled to attend: _____

Occupational Health/Medical Surveillance Enrollment

Date of Enrollment _____ or
 Date Waiver Signed _____

Procedure(s) being performed by this individual. Check all applicable procedures.

- Injections
- Feeding
- Weighing
- Blood Collection
- Euthanasia
- Surgery
- Oral Gavage
- Other, specify: _____

Qualifications/relevant experience. Indicate if this individual has performed the procedure with this species previously?

EUTHANASIA
**[THE ATTENDING VETERINARIAN MUST BE CONSULTED REGARDING
 THE METHOD OF EUTHANASIA PRIOR TO SUBMISSION OF PROTOCOL.]**

This section must be completed for every protocol; even though your study does not involve planned euthanasia. The method outlined may be used in the event of unanticipated injury or illness. Following euthanasia, death should be assured by creating a bilateral pneumothorax, aortic transection, or other certain physical means as appropriate.

INDIVIDUAL(S) PERFORMING EUTHANASIA:

Name Lauren Irick
 Name Harold Farris
 Name Linda Fulton
 Name _____

POSSIBLE METHOD	SPECIES 1	SPECIES 2
<input checked="" type="radio"/> CO ₂ Precharged Chamber	CD Hairless Rat	
<input type="radio"/> Cervical Dislocation under CO ₂ anesthesia		
<input type="radio"/> Decapitation under CO ₂ anesthesia		
<input type="radio"/> Captive Bolt		
<input type="radio"/> Cervical Dislocation*		
<input type="radio"/> Decapitation*		
<input type="radio"/> Injectable euthanasia agents (see dose chart below)		—
<input type="radio"/> Other*		

*Must include justification if the method is not recommended by the AVMA Panel on Euthanasia.

Species	Agent	Dose	Route

**USE OF ANIMALS IN BIOMEDICAL AND
AGRICULTURAL RESEARCH AND TEACHING**

***COMPLETE THOSE SECTIONS RELEVANT TO YOUR PROJECT —
DISCARD ALL SECTIONS NOT APPLICABLE TO YOUR PROJECT***

CHECK APPLICABLE SECTIONS:

- SECTION A **Non-Surgical Procedures Involved**
Experimental procedures including non-surgical, pre-surgical, and post-surgical
- SECTION A **Antibody Production Involved**
- SECTION B **Surgical Procedures Involved**
 - Non-Survival Surgery
 - Survival Surgery
 - Multiple Survival Surgery
- SECTION C **Field Studies Involved**
- SECTION D **Hazardous Agents Involved**
Copy of approval letter from the Institutional Biosafety Committee must be submitted prior to ARC approval.
- SECTION E **Farm Animals Used in Agricultural Teaching and Production**

SECTION B
SURGICAL PROCEDURES

The attending veterinarian must be consulted on anesthetic regimens, surgical procedures and post-surgical care.

1. NON-SURVIVAL SURGERY

Yes
 No

SURVIVAL SURGERY (Any surgical procedure, including biopsies, where an animal is allowed to recover from anesthesia, regardless of the length of survival period. In non-survival surgery, the animal is euthanized while still anesthetized. Reference: Clemson University Animal Care and Use Handbook, Surgery Policy.)

Yes
 No

MULTIPLE SURVIVAL SURGERY (If yes, provide justification for multiple survival surgical procedures.)

Yes
 No

Justification:

2. SURGEON

NAME Linda Fulton
PHONE 656-5034

3. SITE OF HOUSING PRIOR TO AND AFTER SURGERY

BUILDING Godley-Snell Research Center ROOM _____

SURGERY ROOM

BUILDING Godley-Snell Research Center ROOM _____

4. BRIEFLY DESCRIBE SURGICAL PROCEDURE. (Include anticipated duration of procedure from start of anesthesia to recovery.)

A 5 cm skin incision is made 2 cm lateral to the dorsal midline beginning at the level of T-11 or 12 (thoracic vertebra.) A total of 2 incisions are made (one on right side of dorsal midline and one on left side.) One incision is closed using 1 ml Gel Formulation III or IV and skin staples placed every 1 cm. The second incision is closed using nine staples spaced every 0.5 mm.

SECTION B
SURGICAL PROCEDURES

5. HEALTH ASSESSMENT (PRIOR TO SURGERY)

How will health status of animals be assessed before initiation of procedure?

Quarantined for 10 days.

Will animals be fasted prior to surgery?

Yes How long? _____
 No

Will water be withheld prior to surgery? (Water should not be withheld from rodents and should not be withheld for more than six hours for non-rodents.)

Yes How long? _____
 No

Observation	Observation Frequency
<input type="radio"/> Body Temperature	
<input checked="" type="radio"/> Weight	weekly
<input checked="" type="radio"/> Behavior	daily
<input type="radio"/> Other, specify: 	

6. ANESTHETIC PROTOCOL

Anesthetist June Brock, Lauren Irick, or Diane Bailey

	Agent	Dose	Route
Pre-Anesthetic Agent #1	Acpromazine	0.5 mg/kg	subcutaneous
Pre-Anesthetic Agent #2	Buprenorphine	0.05 mg/kg	subcutaneous
Pre-Anesthetic Agent #3			
Anesthetic Agent #1	Isofluorane	1.5 - 2.5 %	Inhalation
Anesthetic Agent #2			
Anesthetic Agent #3			

SECTION B
SURGICAL PROCEDURES

Describe indices and methods to be used to monitor level of anesthesia and condition during surgery. Should be monitored every five minutes. Purposeful movement in response to painful stimuli, including toe pinch, must be abolished before surgery.

- Respiratory Rate
- Heart Rate
- Capillary Refill Time (Mucus Membrane Color)
- Body Temperature
- Reflexes
- Blood Pressure
- O₂ Saturation - Pulse Oximeter
- O₂ Saturation - Blood Gases
- EKG
- PCR/TP

Paralyzing Drug

Yes Drug _____ Dose _____ Route _____
 No

If yes, provide justification:

POST-OPERATIVE RECOVERY

	Agent	Dose	Route
Analgesic Agent #1	Buprenorphine	0.005 - 0.05 mg/kg every 8 - 12 hours for 24 hours.	subcutaneous
Analgesic Agent #2			
Analgesic Agent #3			

If analgesics will not be used, provide justification with references to explain how analgesics will interfere with the proposed research.

What is the anticipated duration of recovery from anesthesia? 30 minutes

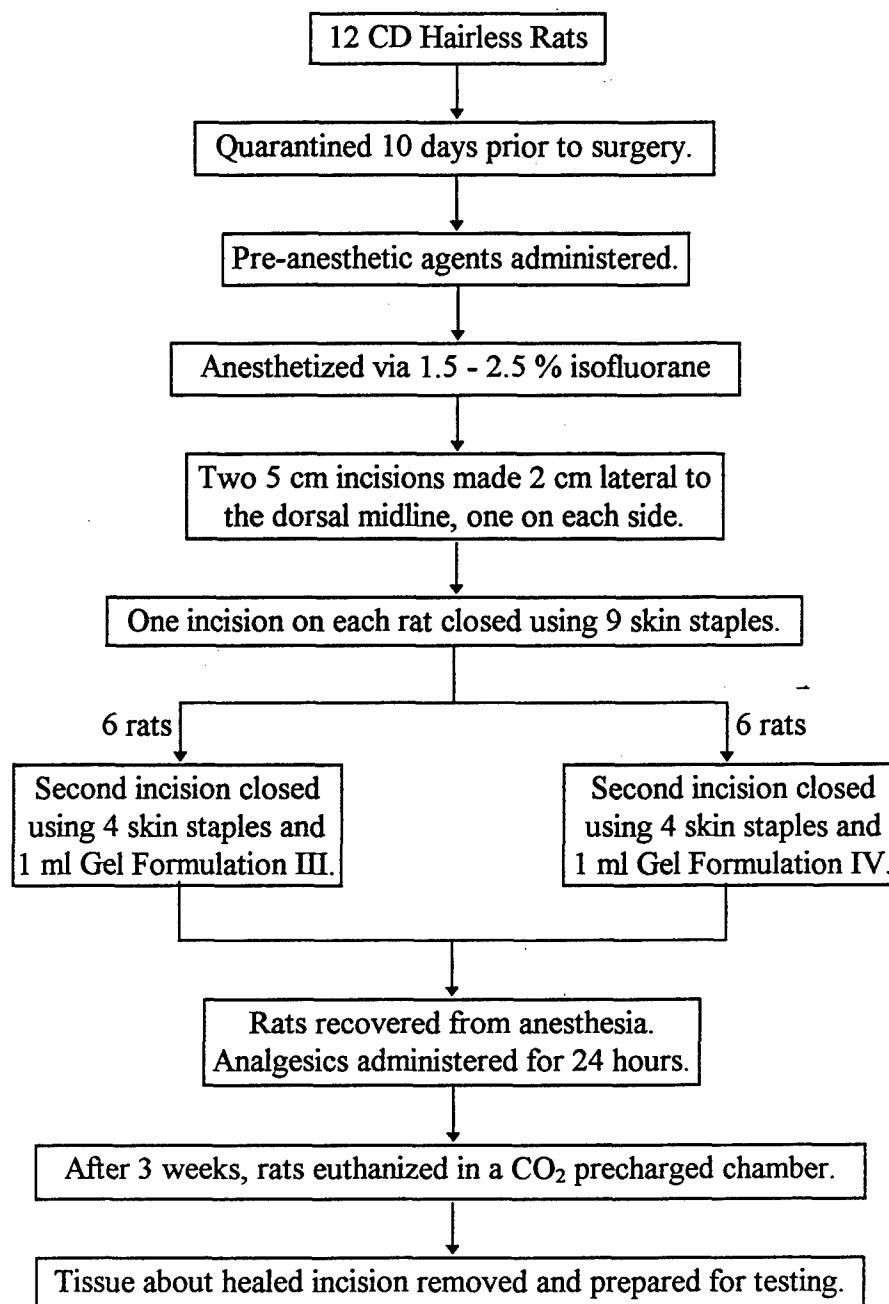
What specifically will be monitored post-operatively?	How often?
<input type="radio"/> Body Temperature	
<input type="radio"/> Weight	
<input checked="" type="radio"/> Behavior	
<input type="radio"/> Other, specify	daily

SECTION B
SURGICAL PROCEDURES

After recovery and during experimental study, what criteria will be used to assess pain, distress and discomfort?	
	How often? Post-surgical observations should be recorded at least three days post-operatively.
● Appetite	daily
● Weight	weekly
● Behavior	daily
● Other, specify: incisions examined for signs of infection or self-induced trauma.	daily

HUMANE ENDPOINTS TO PREVENT CHRONIC PAIN AND DISTRESS	
If these humane endpoints are not appropriate for the study or cannot be used for scientific reasons, scientific justification must be provided for ARC review and inclusion in the USDA Annual Report of Research Facilities.	
● Inappetance > 48 hours	
● Weight loss > 20% of normal weight	
● Mutilation of operative site	
● Depression (48 - 72 hours)	
○ Non-weight bearing > 72 hours	
● Infection not resolved by antimicrobial therapy	
● Moderate to severe clinical signs of pain or distress unalleviated by appropriate analgesic	
○ Other, specify	

Part II: Rat Study, Tissue Adhesive



AN OVERALL ABSTRACT FOR THE THREE PROTOCOLS

Many approaches are being used for treating traumatic and burn wounds such as those encountered in battlefield injuries and burns. However, constraints such as infections, excessive bleeding and/or extreme tissue sensitivity make the treatment of these wounds especially challenging. Thus, the primary objective of this nine-month program is to develop a bioabsorbable (or simply absorbable) hemostatic tissue adhesive with most, if not all, of the following attributes: (1) it can be extruded easily from a syringe as a viscous liquid formulation; (2) the extruded liquid adheres to the tissues and provides sufficient bond strength to keep approximated ends at the wound site in position during healing; (3) the extruded liquid transforms into a gel form at an irregular wound site to allow for 2 to 4 weeks residence time and modulates the oxygen and water vapor transmissions; (4) the extruded system before and after gel formation should be mechanically and chemically compatible with injured tissue and any exposed nerve endings; (5) the formulation can be used for the controlled delivery of antibiotics such as vancomycin; and (6) the selected formulations do not interfere with, and preferably accelerate, wound healing. As a secondary objective, the developed formulations can eventually be used clinically to deliver growth factors for accelerated wound healing. Most pertinent to the three individual protocols is a description of the intended animal studies which can be documented as follows.

Protocol I—Hemostatic Agents

The hemostatic properties of two gel formulations, one with and one without vancomycin, will be evaluated using a rabbit model where liver lacerations will be created using a scalpel. The ability of the gel formulations to stop bleeding will be assessed grossly in terms of time to stop bleeding.

Protocol II—Tissue Adhesives

The adhesive properties of two gel formulations, one with and one without vancomycin, will be evaluated using a set of 6 rats for each formulation. Two 5 cm skin incisions will be made along both sides of the spine. One incision will be closed using a gel formulation and four staples, and the other will be closed using nine staples. After three weeks, the rats will be sacrificed, and the area of skin about the healed incision will be removed and prepared for testing of wound strength. Staples will be removed prior to testing.

Protocol III—Burn Wounds

For burn wound evaluation, an initial study is planned to evaluate the efficacy of a method developed to induce a full thickness thermal injury using a specially designed electrically heated flat plate. This initial evaluation will be a non-survival procedure. Five burn wounds will be created at each side of the rat spine to determine the time needed to induce a full thickness injury. A second animal will be used to evaluate the reproducibility of this method. Once a process is developed for reproducibly inducing full thickness burns, later studies will be planned to evaluate the effect of gel formulations on wound healing.

CLEMSON UNIVERSITY
PROTOCOL FOR USE OF LIVE VERTEBRATES

(SUBMIT THE ORIGINAL AND TWO COPIES TO THE ANIMAL RESEARCH COMMITTEE COORDINATOR, 301H BRACKETT HALL.)

PRINCIPAL INVESTIGATOR	<u>Dr. Shalaby W. Shalaby</u>	PHONE: <u>646-8544</u>
P.I. OF ACTIVITIES AT CLEMSON	<u>Dr. J. David Gangemi</u>	PHONE: <u>656-1440</u>
COLLEGE/UNIT	<hr/>	
DEPARTMENT	<u>Greenville Hospital System/Clemson University Biomedical Cooperative</u>	
PROJECT TITLE	<u>Extrudable Gel-Forming Bioabsorbable Hemostatic Tissue Adhesives for Traumatic and Burn Wounds: Part III— Burn Wounds</u>	

SIGNATURES

As principal investigator, co-investigator, farm manager, or department head/chair, I verify that: (1) the information herein is true and correct and that I am familiar with and will comply with the legal standards of animal care and use established under federal laws, state laws and guidelines, as well as university policies; (2) the proposal has received approval for scientific merit by peer review; and (3) the activities do not unnecessarily duplicate previous experiments. I agree to advise the Animal Research Committee in writing of any changes in the procedures or personnel involved in this project. Such changes will not be implemented until committee approval is obtained. If a change in principal investigator becomes necessary, the committee will be notified immediately.

PRINCIPAL INVESTIGATOR	<u>W. Shalaby</u>	DATE: <u>9/19/96</u>
P.I. OF ACTIVITIES AT CLEMSON	<u>J. David Gangemi</u>	DATE: <u>9/19/96</u>
COLLEGE/UNIT	<hr/>	DATE: <hr/>
DEPARTMENT CHAIR	<u>J. David Gangemi</u>	DATE: <u>9/24/96</u>

Your signature as attending veterinarian verifies that you have: (1) reviewed and are familiar with this proposal; (2) consulted with the principal investigator(s) regarding any surgical procedures and any other procedures that may result in pain or distress; and (3) agree to perform all duties of the attending veterinarian in accordance with The Animal Welfare Act.

ATTENDING VETERINARIAN	<u>H. E. Farris, Jr.</u>	DATE: <u>9-19-96</u>
(SIGNATURE)	<hr/>	<hr/>
TYPED NAME	Harold E. Farris, Jr.	PHONE: <u>656-5034</u>

FOR ARC USE ONLY			
ASSIGNED TO:	<input checked="" type="checkbox"/> FULL COMMITTEE <input type="checkbox"/> DELEGATED	AUP NUMBER	<u>96-053</u>
USDA CATEGORY	<u>—</u>	AMENDMENT NUMBER	<u>1</u>
INSTITUTIONAL CATEGORY	<u>D-2</u>		

ARC APPROVAL:

CHAIR SIGNATURE	<u>Elizabeth Kunkel</u>	DATE: <u>10/14/96</u>
-----------------	-------------------------	-----------------------

GENERAL INFORMATION

1. SUBMISSION TYPE

- New
- Addendum
- Renewal/Continuation (Previous Protocol Number _____)

2. CLASSIFICATION

- Research
- Teaching
- Demonstration
- Farm Management

3. FUNDING SOURCE(S) & GRANT APPLICATION TITLE(S), IF APPLICABLE

U.S. Army Medical Research & Materials Command
Fort Detrick, Frederick, MD 21702-5012

4. COURSE NUMBER(S) AND TITLE(S), IF APPLICABLE

N/A

5. PROJECTED START DATE June 1996 PROJECTED END DATE February 1997

6. ABSTRACT

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- objectives of the research or teaching activity
- species and number of animals
- schedule of the course or the study procedures performed during each phase
- benefits, outcome and results expected

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A flow sheet is required and must be submitted as Attachment 2. Relative information (i.e., numbers of animals, experimental manipulations) should be provided on a flow sheet diagram. The ARC member should be able to follow each manipulation of the animal from initiation to termination.

8. PHS POLICY

PHS policy requires a copy of *Section F from NIH applications* and a copy of all animal methods sections from the proposal. Please provide as an attachment, if applicable. DO NOT SUBMIT THE ENTIRE APPLICATION.

GENERAL INFORMATION
DUPLICATE PAGE AS NEEDED

9. DESCRIPTION OF ANIMALS (COMMON NAMES REQUIRED)

SPECIES: Rat		
BREED/STRAIN: CD Hairless		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input checked="" type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input checked="" type="radio"/> FEMALE	3 mos.	250 g
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments
9	9	

SPECIES:		
BREED/STRAIN:		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input type="radio"/> FEMALE		
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments

SPECIES:		
BREED/STRAIN:		
<input type="radio"/> GROUP HOUSING	IF GROUP, NO. PER CAGE	
<input type="radio"/> SINGLE HOUSING		
<input type="radio"/> MALE	AGE	WEIGHT
<input type="radio"/> FEMALE		
Maximum number maintained at any given time	Total Number Required for Three Year Period	Comments

GENERAL INFORMATION

10. SOURCE OF ANIMALS

- Commercial Vendor Charles River Company, Brookline Massachusetts
- Captured from Wild (A copy of the permit MUST be attached.)
- Transferred from Another Protocol; provide number _____
- Bred or reared at Clemson
- Donated to become Clemson property
- Other: _____

11. METHOD OF ANIMAL IDENTIFICATION

- Cage Cards
- Ear-Punch
- Collars
- Leg Bands
- Wing Tags
- Other Tags
- Tattoos — EAR
- Electronic
- Branding (Freeze)
- Branding (Hot-Iron)
- Other: _____

12. DISPOSAL OF ANIMALS AFTER COMPLETION OF STUDY/PROCEDURE

- Euthanized by methods outlined in the Euthanasia section of this protocol
- Returned to wild
- Returned to production/breeding unit
- Sold
- Transferred protocol number: _____
- Slaughter with conformation to the Humane Slaughter Act
- Other: _____

ALTERNATIVES TO THE USE OF ANIMALS - The Animal Welfare Act requires that the Animal Research Committee must ensure the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to animals, and has provided a written narrative description of the methods and sources.

13. What is the justification for using live animals rather than alternative means of achieving the research goals?

The proposed study requires inducing full thickness burns and monitoring healing over a three week period. Therefore, there is no *in vitro* model to substitute for the *in vivo* one.

GENERAL INFORMATION

14. You are required to conduct a literature search to determine that either (1) there are no alternative methodologies by which to conduct this study, or (2) there are alternative methodologies, but these are not appropriate for your particular study. What procedures, specific databases, and sources did you use to determine that non-painful alternatives were not available or appropriate?

- AGRICOLA
- Animal Welfare Information Center
- Biological Abstracts
- Index Medicus
- National Agricultural Library *Phone: (301) 504-6212*
- Contacted colleagues
- Other: MED-LINE, Chem Abstracts, National Medical Library

What were your findings with respect to alternative methodologies?

No alternatives exist.

15. Why have you selected the particular species proposed in this project?

Lowest possible form of vertebrate.

16. Provide an explanation of how the numbers of animals to be used were derived. Numbers should be based on scientific and statistical requirements to achieve objectives. These numbers must be consistent with those used in the flowsheet. If statistical assistance is needed, contact the Office of Experimental Statistics at 656-3028.

To evaluate each of the three gel formulations, 9 rats are required, three rats for each formulation. Each animal will receive two burns. One burn will be left as an untreated control, the other burn will be treated with a gel formulation. Nine rats represent the minimum number of animals required for data with statistical relevance.

DUPLICATION OF RESEARCH - The Animal Welfare Act requires that the principal investigator provide written assurance that proposed research is not unnecessarily duplicative.

17. Does the proposed research duplicate any previous work?

- Yes
- No

17a. If no, what procedures and sources did you use to determine that the proposed research does not duplicate previous work?

MED-LINE, Chem Abstracts, and the National Medical Library

17b. If yes, provide justification for the need to duplicate previous work.

N/A

QUALIFICATIONS OF PERSONNEL
(Duplicate this page as needed.)

Carefully review the Qualifications of Personnel instruction section located in the front of this form. Complete the following for people who will conduct procedures using animals (especially surgery, anesthesia, pre- or post-operative care, or euthanasia). An occupational health program is mandatory for personnel who work with laboratory animal facilities or have animal contact. The ARC will provide advice and training on all of these procedures if the personnel listed have no previous relevant experience.

List individuals who will have animal contact in association with this protocol and provide the date of their enrollment the Clemson University occupational health program. If individuals listed in this section are not enrolled, they must contact the Nursing and Wellness Center at 656-3076 to enroll or to sign a waiver of exclusion. Individuals not enrolled cannot participate in the study and approval will not be given until enrollment is verified.

FULL NAME: Research Services Personnel

TITLE: _____

Clemson University Animal Care and Use Seminar attendance

Date attended: _____
 Date scheduled to attend: _____

Occupational Health/Medical Surveillance Enrollment

Date of Enrollment _____ or
 Date Waiver Signed _____

Procedure(s) being performed by this individual. Check all applicable procedures.

- Injections
- Feeding
- Weighing
- Blood Collection
- Euthanasia
- Surgery
- Oral Gavage
- Other, specify: _____

Qualifications/relevant experience. Indicate if this individual has performed the procedure with this species previously?

EUTHANASIA**[THE ATTENDING VETERINARIAN MUST BE CONSULTED REGARDING
THE METHOD OF EUTHANASIA PRIOR TO SUBMISSION OF PROTOCOL.]**

This section must be completed for every protocol; even though your study does not involve planned euthanasia. The method outlined may be used in the event of unanticipated injury or illness. Following euthanasia, death should be assured by creating a bilateral pneumothorax, aortic transection, or other certain physical means as appropriate.

INDIVIDUAL(S) PERFORMING EUTHANASIA:

Name Lauren Irick
Name Harold Farris
Name Linda Fulton
Name _____

POSSIBLE METHOD	SPECIES 1	SPECIES 2
<input checked="" type="radio"/> CO ₂ Precharged Chamber	rat	
<input type="radio"/> Cervical Dislocation under CO ₂ anesthesia		
<input type="radio"/> Decapitation under CO ₂ anesthesia		
<input type="radio"/> Captive Bolt		
<input type="radio"/> Cervical Dislocation*		
<input type="radio"/> Decapitation*		
<input type="radio"/> Injectable euthanasia agents (see dose chart below)		
<input type="radio"/> Other*—for non-survival procedure		

*Must include justification if the method is not recommended by the AVMA Panel on Euthanasia.

Species	Agent	Dose	Route

**USE OF ANIMALS IN BIOMEDICAL AND
AGRICULTURAL RESEARCH AND TEACHING**

***COMPLETE THOSE SECTIONS RELEVANT TO YOUR PROJECT —
DISCARD ALL SECTIONS NOT APPLICABLE TO YOUR PROJECT***

CHECK APPLICABLE SECTIONS:

- SECTION A **Non-Surgical Procedures Involved**
Experimental procedures including non-surgical, pre-surgical, and post-surgical
- SECTION A **Antibody Production Involved**
- SECTION B **Surgical Procedures Involved**
 - Non-Survival Surgery
 - Survival Surgery
 - Multiple Survival Surgery
- SECTION C **Field Studies Involved**
- SECTION D **Hazardous Agents Involved**
Copy of approval letter from the Institutional Biosafety Committee must be submitted prior to ARC approval.
- SECTION E **Farm Animals Used in Agricultural Teaching and Production**

SECTION B
SURGICAL PROCEDURES

The attending veterinarian must be consulted on anesthetic regimens, surgical procedures and post-surgical care.

1. NON-SURVIVAL SURGERY

Yes
 No

SURVIVAL SURGERY (Any surgical procedure, including biopsies, where an animal is allowed to recover from anesthesia, regardless of the length of survival period. In non-survival surgery, the animal is euthanized while still anesthetized. Reference: Clemson University Animal Care and Use Handbook, Surgery Policy.)

Yes
 No

MULTIPLE SURVIVAL SURGERY (If yes, provide justification for multiple survival surgical procedures.)

Yes
 No

2. SURGEON

NAME Linda Fulton

PHONE 656-5034

3. SITE OF HOUSING PRIOR TO AND AFTER SURGERY

BUILDING Godley-Snell Research Center ROOM

SURGERY ROOM

BUILDING Godley-Snell Research Center ROOM

4. BRIEFLY DESCRIBE SURGICAL PROCEDURE. (Include anticipated duration of procedure from start of anesthesia to recovery.)

Two burns will be induced on each rat following a protocol developed in an earlier pilot study. A 0.25 in² electrically heated flat plate will be used to induce full thickness burns. Skin lateral to the dorsal midline beginning at the level of T-11 or 12 (thoracic vertebra) will be elevated, clamped with forceps, and contacted with a flat plate at 100°C for 20 seconds. One burn per animal will receive one of three gel formulations: Formulation I will be a placebo; Formulation II will contain antibiotics; Formulation III will contain growth promoters.

SECTION B
SURGICAL PROCEDURES

5. HEALTH ASSESSMENT (PRIOR TO SURGERY)

How will health status of animals be assessed before initiation of procedure?

Quarantined for 10 days prior to procedure.

Will animals be fasted prior to surgery?

Yes How long? _____
 No

Will water be withheld prior to surgery? (Water should not be withheld from rodents and should not be withheld for more than six hours for non-rodents.)

Yes How long? _____
 No

Observation	Observation Frequency
<input type="radio"/> Body Temperature	
<input checked="" type="radio"/> Weight	weekly
<input checked="" type="radio"/> Behavior	daily
<input type="radio"/> Other, specify: 	

6. ANESTHETIC PROTOCOL

Anesthetist June Brock, Lauren Irick, or Diane Bailey

Agent	Dose	Route
Pre-Anesthetic Agent #1	Acepromazine 0.5 mg/kg	subcutaneous
Pre-Anesthetic Agent #2	Buprenorphine 0.05 mg/kg	subcutaneous
Pre-Anesthetic Agent #3		
Anesthetic Agent #1	Isofluorane To Effect (1 - 2.5 %)	Inhalation
Anesthetic Agent #2		
Anesthetic Agent #3		

SECTION B
SURGICAL PROCEDURES

Describe indices and methods to be used to monitor level of anesthesia and condition during surgery. Should be monitored every five minutes. Purposeful movement in response to painful stimuli, including toe pinch, must be abolished before surgery.

- Respiratory Rate
- Heart Rate
- Capillary Refill Time (Mucus Membrane Color)
- Body Temperature
- Reflexes
- Blood Pressure
- O₂ Saturation - Pulse Oximeter
- O₂ Saturation - Blood Gases
- EKG
- PCR/TP

Paralyzing Drug

Yes Drug _____ Dose _____ Route _____
 No

If yes, provide justification:

POST-OPERATIVE RECOVERY— N/A, Non-Survival Surgery

	Agent	Dose	Route
Analgesic Agent #1	Buprenorphine	0.005 - 0.05 mg/kg every 8 - 12 hours for 24 hours.	subcutaneous
Analgesic Agent #2			
Analgesic Agent #3			

If analgesics will not be used, provide justification with references to explain how analgesics will interfere with the proposed research.

What is the anticipated duration of recovery from anesthesia?

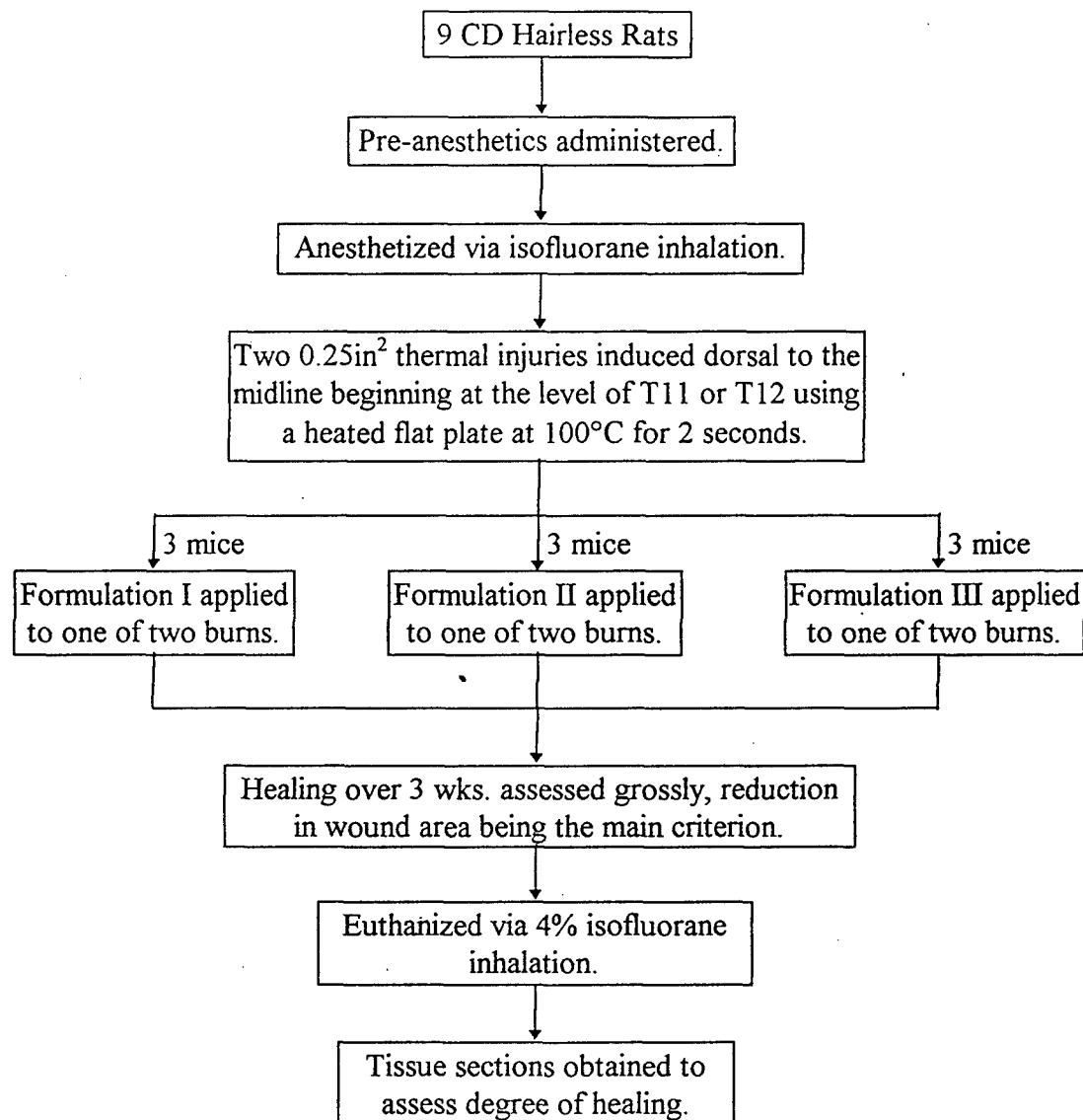
What specifically will be monitored post-operatively?	How often?
<input type="radio"/> Body Temperature	
<input type="radio"/> Weight	
<input checked="" type="radio"/> Behavior	daily
<input type="radio"/> Other, specify:	

SECTION B
SURGICAL PROCEDURES

<p>After recovery and during experimental study, what criteria will be used to assess pain, distress and discomfort?</p> <ul style="list-style-type: none"> ● Appetite ● Weight ● Behavior ● Other, specify: incisions examined for signs of infection or self-induced trauma. 	<p>How often?</p> <p>Post-surgical observations should be recorded at least three days post-operatively.</p> <p>daily</p> <p>weekly</p> <p>daily</p> <p>daily</p>
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HUMANE ENDPOINTS TO PREVENT CHRONIC PAIN AND DISTRESS	
If these humane endpoints are not appropriate for the study or cannot be used for scientific reasons, scientific justification must be provided for ARC review and inclusion in the USDA Annual Report of Research Facilities.	
<ul style="list-style-type: none"> ● Inappetance > 48 hours ● Weight loss > 20% of normal weight ● Mutilation of operative site ● Depression (48 - 72 hours) <input type="radio"/> Non-weight bearing > 72 hours ● Infection not resolved by antimicrobial therapy ● Moderate to severe clinical signs of pain or distress unalleviated by appropriate analgesic <input type="radio"/> Other, specify 	

Part III: Rats, Burn Wounds



AN OVERALL ABSTRACT FOR THE THREE PROTOCOLS

Many approaches are being used for treating traumatic and burn wounds such as those encountered in battlefield injuries and burns. However, constraints such as infections, excessive bleeding and/or extreme tissue sensitivity make the treatment of these wounds especially challenging. Thus, the primary objective of this nine-month program is to develop a bioabsorbable (or simply absorbable) hemostatic tissue adhesive with most, if not all, of the following attributes: (1) it can be extruded easily from a syringe as a viscous liquid formulation; (2) the extruded liquid adheres to the tissues and provides sufficient bond strength to keep approximated ends at the wound site in position during healing; (3) the extruded liquid transforms into a gel form at an irregular wound site to allow for 2 to 4 weeks residence time and modulates the oxygen and water vapor transmissions; (4) the extruded system before and after gel formation should be mechanically and chemically compatible with injured tissue and any exposed nerve endings; (5) the formulation can be used for the controlled delivery of antibiotics such as vancomycin; and (6) the selected formulations do not interfere with, and preferably accelerate, wound healing. As a secondary objective, the developed formulations can eventually be used clinically to deliver growth factors for accelerated wound healing. Most pertinent to the three individual protocols is a description of the intended animal studies which can be documented as follows.

Protocol I—Hemostatic Agents

The hemostatic properties of two gel formulations, one with and one without vancomycin, will be evaluated using a rabbit model where liver lacerations will be created using a scalpel. The ability of the gel formulations to stop bleeding will be assessed grossly in terms of time to stop bleeding.

Protocol II—Tissue Adhesives

The adhesive properties of two gel formulations, one with and one without vancomycin, will be evaluated using a set of 6 rats for each formulation. Two 5 cm skin incisions will be made along both sides of the spine. One incision will be closed using a gel formulation and four staples, and the other will be closed using nine staples. After three weeks, the rats will be sacrificed, and the area of skin about the healed incision will be removed and prepared for testing of wound strength. Staples will be removed prior to testing.

Protocol III—Burn Wounds

For burn wound evaluation, a full thickness thermal injury will be achieved using a specially designed electrically heated flat plate. Burn wounds will be created at two sides of the rat spine. One burn will be left untreated for control and one will be treated with one of three gel formulations. Nine animals will be used to test the experimental gel formulations, i.e., three animals per gel formulation. The extent of healing over a period of three weeks will be assessed grossly, with reduction in wound area being the main criterion.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

10 Aug 98

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following contracts. Request the limited distribution statement for these contracts be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

<u>Contract Number</u>	<u>Accession Document Number</u>
DAMD17-91-C-1020	ADB187724 +✓
DAMD17-92-C-2053	ADB196427 +
DAMD17-94-C-4022	ADB190750 +
DAMD17-94-C-4023	ADB188373 +
DAMD17-94-C-4027	ADB196161 +✓
DAMD17-94-C-4029	ADB190899 +
DAMD17-94-C-4039	ADB188023 +
DAMD17-94-C-4024	ADB189184 +
DAMD17-94-C-4026	ADB187918 +
DAMD17-94-J-4250	ADB221970
DAMD17-94-J-4250	ADB230700
DAMD17-96-1-6241	ADB233224
DAMD17-96-1-6241	ADB218632 ✓
DAMD17-94-J-4496	ADB225269
DAMD17-94-J-4392	ADB225308
DAMD17-94-J-4455	ADB225784
DAMD17-94-J-4309	ADB228198
DAMD17-91-C-1135	ADB233658
DAMD17-94-J-4038	ADB232313
DAMD17-94-J-4073	ADB222794
DAMD17-94-J-4131	ADB219168
DAMD17-94-J-4159	ADB232305
MIPR- 95MM5535	ADB232218
95MM5605	ADB233374
95MM5673	ADB226037

MCMR-RMI-S

SUBJECT: Request Change in Distribution Statement

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or email: judy_pawlus@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:


PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management